DOCKET NO.: CARP-0057 **PATENT**

APPENDIX A

Claim Limitation	Support in Queen Priority Applications
1. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains,	
which humanized immunoglobulin specifically binds to an antigen with an affinity constant **of at least 10^7 M^{-1}**	No. Limitation in bold not recited in two earliest priority applications; recitation sets a different floor for affinity than recited in the priority applications. These applications recite binding affinities "stronger than about 10^8 M^{-1}" (page 4, line 36 of Queen '252 and page 4, line 25 of Queen '975) and "of at least about 10^8 M^{-1}, preferably 10^9 M^{-1} to 10^{10} M^{-1}, or stronger" (page 7, lines 13-14 of Queen '252 and page 8, lines 4-5 of Queen '975). The claims of both applications recite an affinity of 10^8 M^{-1}. The affinities of the example antibodies are not provided; all that is stated is that they have approximately the same affinity as the donor. The affinity of the donor antibody is not provided.

and **no greater than about four-fold** that of the donor immunoglobulin,	No. Limitation not present in two earliest priority applications. Rather, Queen '252 recites that affinity levels "may be within about 4 fold of the donor immunoglobulin's original affinity to the antigen" (page 4, line 36-37). The background of Queen '252 focuses upon the problem of humanization procedures that result in a loss of affinity for the antigen of at least 2 to 3-fold, and as much as 10-fold (page 2, lines 30-35). Recitation in Queen '252 was, thus, setting a floor for affinity, not a ceiling. The recitation in the issued claims is setting a ceiling. No similar recitation is present in Queen '975.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework **outside the Kabat and Chothia CDRs**,	No. Limitation not present in two earliest priority applications. Neither application specifies that the antibodies must comprise residues from the donor framework outside the Kabat and Chothia CDRs. CDRs and frameworks are defined in terms of Kabat in both priority applications (page 8, lines 21-25 of Queen '252 and page 21, lines 19-22 of Queen '975). There is no specific reference to or definition of Chothia CDRs. The claims of Queen '252 recite that only a single residue need be changed (claim 8). Two residues indicated as changed in the lone example are residues 27 and 30 of the heavy chain (see Figure 1). Both are within a Chothia CDR. Queen '975 recites that the donor residue can be chosen if it falls into one or more of four different categories (see page 21, lines 19-34). Two residues indicated as changed in the lone example are residues 27 and 30 of the heavy chain (see Figure 1), both of which are within a Chothia CDR.

wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	
and each of said donor amino acids: (I) is adjacent to a CDR in the donor immunoglobulin sequence or	
(II) contains an atom within a distance of 4 Å of a CDR in said humanized immunoglobulin.	No. Limitation not present in two earliest priority application. No distance is recited in Queen '975. Queen '252 recites a distance of "3 angstroms" (page 13, line 1). The distance has been extended in the claims as issued.